COLONOSCOPIC SCREENING SHOWS INCREASED EARLY INCIDENCE AND PROGRESSION OF ADENOMAS IN CYSTIC FIBROSIS

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Abstract

Background. Colorectal cancer is an emerging problem in cystic fibrosis (CF). The goal of this study was to evaluate adenoma detection by systematic colonoscopic screening and surveillance.

Methods. We analyzed prospectively collected results of colonoscopies initiated at age 40 years from 88 CF patients at a single Cystic Fibrosis Center. We also reviewed results of diagnostic colonoscopies from 27 patients aged 30-39 years performed during the same time period at the Center.

Results. The incidence of polyp detection increased markedly after age 40 in CF patients. Greater than 50% were found to have adenomatous polyps; approximately 25% had advanced adenomas as defined by size and/or histopathology; 3% were found to have colon cancer. Multivariate analysis demonstrated specific risk factors for adenoma formation and progression.

Conclusions. Early screening and more frequent surveillance should be considered in patients with CF due to early incidence and progression of adenomas in this patient population.

Key Words. Cystic fibrosis; adenoma; colon cancer; colorectal cancer screening

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Introduction

Cystic fibrosis (CF) is the most common life-limiting autosomal recessive genetic disease affecting Caucasians. Individuals with CF suffer from complications in multiple organ systems, including the respiratory, gastrointestinal, and reproductive tracts. Pulmonary complications continue to be the leading cause of morbidity and mortality. Fortunately, early detection, improvements in nutrition, bronchial clearance therapy, and organ transplantation have led to a marked increase in life expectancy over the past 30 years. Average life expectancy today is reaching nearly 50 years of age [1]. With increasing survival, new complications, especially those involving the gastrointestinal tract are being identified. Adult gastroenterologists are increasingly involved in the care of multiple gastrointestinal complications associated with CF [2].

Gastrointestinal malignancies, particularly colon cancer, are emerging problems in CF patients [3]. This issue was first described in detail in an epidemiological review of national CF registry data from 1990 through 2009. Maisonneuve, et al.discovered an increased incidence of gastrointestinal malignancy in CF patients that was nearly seven times that of the national average. [3] While there was an increased risk of malignancy in CF patients of all ages, the highest incidence appeared in the 5th decade of life where 11 cases were identified in 10,751 patient/years, an 8.7 fold increase from the expected

incidence rate [3]. The majority of gastrointestinal malignancies discovered, 28 of 45, were colorectal cancers [3].

Early detection of colorectal cancers and removal of adenomatous polyps reduces colon cancer mortality [4-6]. There is now broad consensus endorsing population-wide colon screening, generally starting at age 50 for average risk individuals and significantly earlier in the presence of genetic risk factors [7-10]. Given the early and increased risk of colon cancer in individuals with CF, alternative screening strategies are needed to prevent colorectal cancer mortality. Guidelines or recommendations have not yet been provided by any medical societies on the appropriate age for initiation of screening and intervals for re-screening and surveillance in CF patients. Systematic outcomes data are critically needed for development of such guidelines. In addition, specific clinical circumstances need to be considered in CF individuals, including the patient's overall medical condition, evaluation for organ transplantation, and use of immunosuppressive medications when developing these guidelines.

This project sought to generate outcomes data in effort to facilitate the establishment of screening and surveillance guidelines in CF patients. Specifically, this project sought to determine the incidence of colorectal polyp and advanced adenoma formation in an adult CF population. Additionally, this project sought to determine the age of onset of colorectal polyp formation and to determine which specific clinical factors should be considered when developing colorectal screening guidelines for CF patients.

The University of Minnesota Cystic Fibrosis Center has maintained a systematic colorectal screening program for CF patients starting at the age of 40 for the last seven years. The results of a study of 45 patients enrolled in this program were published in 2014 [11]. Study findings demonstrated an increased rate of adenomatous polyp formation in a cohort of patients in their 40's [11]. Additionally, researchers discovered a sex difference in polyp formation, with a significantly higher number of adenomatous polyps, including high-risk adenomatous polyps, in males than in females undergoing colonoscopy at our center [11].

In order to further validate these results and to explore additional clinical factors associated with polyp development, our research team undertook this project. We aimed to analyze factors that were most likely to discriminate between polyp and non-polyp formers. To determine which factors these were, we analyzed patient characteristics that are associated with increased severity of cystic fibrosis such as having a delta F508 homozygote genotype. In addition, we analyzed patients with CF-related complications more typical in advanced disease, including pancreatic insufficiency and CF-related diabetes, and individuals who required lung transplantation, which may further increase colon cancer risk due to the subsequent immunosuppression. We chose to include patients who would be affected most severely by CF based on the hypothesis that polyps would manifest most abundantly in those affected most severely.

Our site continued to use these screening protocol after our 2014 publication, and these results informed our practice. Since our 2014 publication, we have doubled the number of patients screened at our center, allowing us to collect the amount of data necessary to further test our hypothesis. This paper updates and expands our 2014 research on screening, re-screening, and surveillance colonoscopy results from the same program. In addition, we report results of diagnostic colonoscopic examinations in patients aged 30-39.

Methods

Study design

Results of colonoscopies in CF patients were prospectively collected at the Minnesota Cystic Fibrosis Center between 2008 and 2015, although the formal colorectal cancer screening program was started at the Center in January 2010, at which time all patients followed at the Center began receiving uniform recommendations for screening. Screening colonoscopies performed prior to 2010 were requested by individual physicians concerned about increased prevalence of colon cancer in the CF population. The majority of examinations completed at the Minnesota Cystic Fibrosis Center were performed following administration of a CF-specific colonoscopy preparation [11], but results of

colonoscopies performed at outside institutions were also included. The qualifying criteria for recommendation of a screening colonoscopy were age ≥ 40 years, FEV_1 ≥ 40% predicted, and absence of other contraindications to endoscopic procedures. Individuals with FEV1 < 40% could still be considered for colonoscopic examinations depending on the individual case assessment in coordination with their primary pulmonologist. Re-screening after negative examinations was recommended after three years. Surveillance examination intervals were determined by the colonoscopist based on the number and histopathology of the polyps. Adenomatous polyps were classified to have advanced pathology if they were > 1 cm in size, had villous features, or were noted to have high-grade dysplasia or carcinoma [10]. In addition, we reviewed adenoma detection rates in colonoscopies performed for various diagnostic indications in patients aged 30 years and above. All patients in this study consented to have their data collected and used for research. The study was approved by the Institutional Review Board at the University of Minnesota.

Statistical analysis

Statistical analysis was performed using *R* statistical software version 3.1.2. Subject age at time of initial colonoscopy versus age of subjects not receiving colonoscopy was compared as a continuous variable by T-testing. For comparison of subjects with and without polyps, chi-square analysis was

performed using the categorical variables of sex, history of pancreatic insufficiency, history of cystic fibrosis related diabetes (CFRD), history of distal intestinal obstruction syndrome (DIOS), and history of lung transplant. Age was also compared as a categorical variable for subjects <50 or age ≥50. CF genotype was compared as a categorical variable with ΔF508 homozygotes versus all other genotypes. For multivariate analysis logistic regression analysis was used on the above categorical variables. A p-value of <0.05 was considered to be a significant association.

Results

Patient characteristics

At the time of analysis 111 patients over age 40 were enrolled at the Minnesota Cystic Fibrosis Center Database (Table 1). Of these, 82 had undergone at least one colonoscopy and 32 patients had at least one subsequent colonoscopy. A total of 88 patients have undergone colonoscopies since 2008, including 6 patients who were no longer in the database of active patients because of death or relocation. One subject had a history of familial polyposis and was excluded from factor analysis. There were no observed differences in the clinical

characteristics of age, gender, CF genotype, history of diabetes or pancreatic insufficiency, DIOS, or history of lung transplantation between those who did or did not receive colonoscopic examination.

Adenoma detection on screening and re-screening colonoscopies

Adenomatous polyps were detected on initial screening colonoscopies in 43/88 patients (49%). In addition, 15 patients with negative initial examinations had undergone follow-up re-screening within a mean period of 49 months. Seven (47%) re-screening examinations revealed adenomas, and three of these examinations showed advanced adenomas. Overall, advanced adenomas were found in 20/88 patients (23%). Three or more adenomas and/or advanced histopathology were found in 28/88 patients (32%). Carcinomas were found in 3/88 patients; two of these neoplasms were in situ carcinomas within large polyps that were successfully removed endoscopically. One patient, a ΔF508 homozygote, was found to have invasive rectal carcinoma within the first year following lung transplantation during her first colonoscopy. She ultimately died of complications related to treatments of the cancer.

Patient factors associated with polyp formation

In order to explore specific patient factors that may be associated with polyp formation in our cohort we examined age, gender, homozygous ΔF508 mutation, pancreatic insufficiency, CFRD, DIOS, and history of lung transplantation using univariate analysis (Supplemental Table 1). We found CFRD and homozygous ΔF508 mutation to be statistically significant risk factors, and identified a trend toward significance with lung transplantation. It is important to note that the average time from transplantation to first colonoscopy in this cohort was 4.16 years. CFRD continued to be an independent risk factor on multivariate analysis (Table 2).

Patient factors associated with multiple or high risk polyp formation

Previously, we found male sex to be associated with greater risk of adenomatous polyp formation in cystic fibrosis [11]. This continued to be a trend in our analyses in this larger cohort. Male sex remained a statistically significant risk factor for the presence of either advanced or multiple adenomas (Table 3). Lung transplantation was also statistically associated with the development of multiple or advanced adenomatous polyps. All three cases of colon cancer were found in patients who were ΔF508 homozygotes with history of CFRD. Their ages ranged

between 47 and 51 at the time of diagnosis. One of these patients was female and a lung transplant recipient, as noted above.

Surveillance colonoscopy adenoma detection

Patients found to have adenomatous polyps on their initial colonoscopies were generally recommended to have repeat examinations within one to two years if they remained medically stable. The majority 13/16 (81%) of these surveillance colonoscopies continued to be positive for adenomatous polyps with 6/16 (38%) having advanced histopathology on subsequent colonoscopies (Table 4).

Diagnostic colonoscopy adenoma detection (age 30-39).

We found 27 patients with CF in our database, age 30-39, who underwent diagnostic colonoscopies. The most common indications for these colonoscopies were hematochezia (n = 7) and abdominal pain (n = 7). Other indications included screening during transplant evaluation (n = 3), iron deficiency anemia (n = 2), persistent diarrhea (n = 2), colitis (n = 1), early family history of colon cancer (n = 1), pseudomembranous colitis (n = 1), and abnormal radiologic imaging with suspected polyp (n = 1). Indications were not stated in 2 colonoscopies. Four of these patients (15%) had adenomatous polyps noted and an advanced adenoma was documented in one of these examinations (4%).

Discussion

Improved life expectancy of CF patients over the past several decades has been accompanied by emergence of new challenges associated with older age. These include digestive tract malignancies, which are present at higher prevalence in CF patients [3]. Of these, colon cancer is the most common, and it is arguably the most preventable with an efficient screening and surveillance program. Most colon cancers arise from adenomatous polyps, which progress into carcinomas as they become larger and accumulate mutations permissive to malignant transformation. Early detection of premalignant adenomas and localized carcinoma can prevent cancer and cancer-related deaths. This is the underlying principle behind colon cancer screening in the general population, which is typically initiated at age 50. There are currently no specific guidelines for colon cancer screening that consider an increased risk associated with CF, although these are being considered by the National Cystic Fibrosis Foundation in collaboration with the American Gastroenterological Association. Paucity of data specific to CF patients continues to be a major challenge for these efforts.

The Minnesota Cystic Fibrosis Center has maintained a systematic colonoscopic screening program for a number of years for patients age 40 or above. Our results demonstrate that approximately one quarter of these patients have advanced neoplasms in the colon. This is at least 5 times higher than the

2-5% reported in different studies for the general population 40-49 years old [12-14]. Obviously, these numbers alone do not predict the fraction of patients that would progress to incurable cancer prior to another fatal CF-related complication. However, it is notable that three patients (3%) in this study were found to have carcinomas. It is likely that the two individuals who had carcinoma in situ were spared from dealing with a more advanced stage of cancer because of early detection.

In the general population and in recognized hereditary colon cancer syndromes the incidence of colon cancer rises steadily with age [14]. This appears to be true also in CF patients. We found that the rate of colon adenomas and advanced neoplasias was significantly lower in patients 30-39 years of age compared to our screening cohort of patients 40 years of age or older. Remarkably, the rate of advanced neoplasia formation in CF patients 40-49 years of age is comparable to that of nonagenarians in the general population [15]. An accelerated rate of polyp development and growth associated with CF is further suggested by the high detection rate of new adenomas, including advanced polyps, on surveillance and re-screening colonoscopies performed at relatively short intervals in our program despite optimized colon preparations that should have minimized missed polyps [11]. Taken together our data suggest a significant shift in adenoma formation and progression at a younger age in patients with CF.

The underlying biology driving colon carcinogenesis remains speculative. The cystic fibrosis transmembrane conductance regulator (CFTR) was identified as a potential driver of colorectal carcinoma in a forward-based genetic screen [16]. The major recognized function of CFTR is that of an anion channel in the epithelial cells, and its deficiency decreased the level of hydration of the mucus layer. Stagnant mucus is associated with bacterial overgrowth in *Cftr*-deficient mice along with dysregulation in gene expression involved in inflammation and epithelial homeostasis [17, 18]. These physical changes in the mucus layer are also associated with compositional changes in microbiota, which can further affect epithelial function [19-22]. Interestingly, the pattern of dysregulated genes associated with CFTR deficiency overlaps with that observed following deletion of *Kcnq1*, which encodes for a potassium channel that is linked to CFTR function and is another tumor suppressor gene associated with gastrointestinal malignancies [23]. However, it is also important to recognize that CFTR plays important roles in epithelial biology beyond being a mere anion channel. It complexes with cytoskeletal elements, associates with protein kinases, participates in maintenance of tight junctions, and may contribute to epithelial cell polarization [24-26]. Disruption of these functions can further contribute to cancer development and progression. In fact, the *CFTR* gene has been reported to be commonly hypermethylated in different cancer cell lines and tumor samples, suggesting a role for CFTR dysfunction in colon cancer in the general population as well [27-31].

Our attempt at identifying specific clinical variables, although certainly limited by the small size of the study, does not shed significant light on colon cancer pathogenesis in CF patients. Our finding of male sex association with more advanced polyp histopathology is consistent with the excess colon cancer risk reported previously in the CF population [3] and recapitulates data obtained in the general population [14, 32]. Greater incidence of adenomas in patients with CFRD and ΔF508 homozygocity may be a reflection of a more severe CF phenotype. Our results demonstrating an association between more advanced adenomas and organ transplantation-related immunosuppression are consistent with the augmentation of colon cancer risk in these patients found in epidemiologic studies. However, all of the subpopulations of CF patients remained at significantly increased risk of developing advanced adenomas, and by extension colon cancer.

Emergence of colon cancer as a potentially significant clinical problem in the care of CF patients is a consequence of their improved life expectancy under modern medical care. Further increases in survival should be expected with introduction of new treatments entering clinical practice. Therefore, it is increasingly important to recognize that CF is also a colon cancer syndrome, which deserves special considerations for screening and surveillance. Our data support initiation of colorectal cancer screening at age 40 in medically stable patients. The rate of adenoma detection appears to be significantly lower at

younger ages, although it should be noted that cases of colon cancer associated with CF have been reported even in teenage years.

Currently, we recommend our patients have a re-screening or surveillance colonoscopy in three years if their most recent examination showed less than three polyps without advanced histopathology. This recommendation is based on the high rate of colon neoplasms during such examinations at relatively short intervals. We recommend surveillance colonoscopy after one year in patients found to have three or more polyps or polyps with advanced histopathology. The colonoscopic method of screening is preferred because of the high likelihood of polyp detection and associated polypectomy. However, it may be reasonable to consider at least a fecal immunochemical test (FIT) for patients with poor lung function undergoing a workup for transplantation. Premature colon cancer related death in an organ transplant recipient is tragic for the patient who may have undergone a difficult surgical procedure only to face another major crisis. In addition, detection of colon cancer in a transplant candidate is an important consideration in terms of organ allocation given limited organ availability. If the FIT test is positive, a colonoscopy can still be done usually despite the somewhat increased risk. If negative, colonoscopic screening can be postponed until after transplantation.

In summary, our study confirms the concern for earlier development of colon cancer in CF patients arising via the classic adenoma growth and progression. Increasing life expectancy of CF patients necessitates focused

attention on this emerging problem and development of dedicated recommendations. Limitations of this study include its small sample size and single center experience. Further research is needed and can be aided by careful and detailed entry of neoplasm detection in regional and national CF registries.

Areas for Future Research

This research brought to light several projects necessary to advance the field. National guidelines for colorectal cancer screening are needed to standardized patient care and produce replicable outcomes across clinical sites. Once national guidelines for colorectal cancer screening are established, the resulting influx of screening results into the national CF registries will provide larger patient cohorts for epidemiological research. The knowledge gained through large-scale retrospective studies of this data has the potential to lead to the development of more specific guidelines tailoring preventative efforts to patients most in need, while minimizing the risk of extraneous procedures. With this goal in mind, the Cystic Fibrosis Center at the University of Minnesota continues to record outcomes data from all colonoscopies performed on Center patients.

Additionally, the use of alternative screening modalities besides colonoscopy may be efficacious, but has not been systematically studied in patients with Cystic Fibrosis. The testing and validation of non-invasive diagnostic modalities, such as fecal immunochemical testing, might result in an alternative diagnostic strategy for subjects who are at higher risk for complications from conscious sedation or who decline routine colonscopic screening. Non-invasive screening techniques might also lead to an overall reduction in the costs of screening and increased patient compliance. At the University of Minnesota Cystic Fibrosis Center, we have begun using FIT testing as part of our pre-lung transplant evaluation. An analysis of the results of this FIT testing data is planned in order to determine its correlation with adenomatous polyp discovery on colonoscopy.

Finally, further exploration of the cause of colonic polyp development in cystic fibrosis patients is an additional opportunity to decrease the incidence and burden of disease. While an epidemiological link has been well established, researchers have yet to identify a definitive mechanism of early polyp development in cystic fibrosis patients despite investigations of the previously discussed potential mechanisms. Our Center is using RNA sequencing analysis to study the colonic mucosal lining of individuals with cystic fibrosis in order to identify genes that may be activated in the CF colon that are potentially related to inflammatory pathways identified in colorectal carcinogenesis. With this data, we hope to identify targets for further basic and translational research projects in the

future. A multitude of research projects are necessary to further the knowledge of colorectal cancer development and screening in cystic fibrosis patients, and as the life expectancy of CF patients continues to increase, this research will become even more imperative.

Table 1. Characteristics of 88 patients over the age of 40 with screening colonoscopy

Patient Factor	Patients with At Least 1 Colonoscopy (N=88) Number of patients (percentage)
Age at First Colonoscopy	46.22 years (average)
Male Sex	45 (51%)
Delta F508 Homozygote	38 (43%)
Pancreatic Insufficiency	70 (80%)
CFRD	50 (57%)
DIOS	18 (20%)
Lung Transplant	24 (27%)
Polyp (any colonoscopy)	49 (56%)
Polyp on 1st colonoscopy	43 (49%)
Advanced Pathology (any colonoscopy)	20 (23%)
3 or more polyps (any colonoscopy)	20 (23%)
Advanced Pathology and or 3 or more polyps (any colonoscopy)	28 (32%)
Colon Cancer	3 (3%)

Table 2. Multivariable regression analysis of polyp vs. non-polyp formers

Variable	Polyps present (48 patients)	Polyps absent (39 patients)	Adjusted OR (95% CI)	P Value
Age >50	19 (40%)	10 (28%)	1.82 (0.64-5.17)	0.2628
Male Sex	27 (56%)	17 (44%)	2.18 (0.77-6.17)	0.1411
ΔF508 Homozygote	27** (57%)	11 (28%)	3.88 (1.23-12.24)	0.0206*
Pancreatic Insufficiency	40 (83%)	29 (74%)	0.46 (0.12-1.80)	0.2628
CFRD	35 (73%)	15 (38%)	4.15 (1.29-13.40)	0.0171*
DIOS	9 (19%)	9 (23%)	0.42 (0.12-1.43)	0.1641
Lung Transplant	17 (35%)	7 (18%)	2.00 (0.58-6.89)	0.2715

*P<0.05

**One patient with polyps had no genotype information available and was excluded from the analysis.

Table 3. Multivariate analysis of patient attributes association with ≥ three or high-risk polyp formation

Variable	Polyps present (27 patients)	Polyps absent (60 patients)	Adjusted OR (95% CI)	P Value
Age >50	11 (41%)	19 (32%)	1.36 (0.47-4.02)	0.5685
Male Sex	19 (70%)	25 (42%)	4.51 (1.37-14.88)	0.0133*
ΔF508 Homozygote	15** (58%)	23 (39%)	1.66 (0.53-5.18)	0.3842
Pancreatic Insufficiency	25 (93%)	44 (73%)	2.02 (0.33-12.45)	0.44927
CFRD	20 (74%)	30 (50%)	1.67 (0.49-5.67)	0.41357
DIOS	7 (26%)	11 (18%)	1.25 (0.36-4.38)	0.72154
Lung Transplant	12 (44%)	12 (20%)	3.90 (1.10-13.82)	0.03501*

*P<0.05

**One patient with polyps had no genotype information available and was excluded from the analysis.

Table 4. Results of surveillance colonoscopies, 16 individual patients

Patient	Number of polyps on initial colonoscopy	Number of polyps on follow-up examinations						
		1 year	2 years	3 years	4 years	5 years	6 years	7 years
1	11*	4*	1					
2	10*	6*	9		7*		1	
3	8*		2*					
4	7*	1		7				
5	7*	0						
6	6*	3						
7	5	2		2*		3		
8	4	4	5	5*	6*			
9	4	2						
10	4		1					
11	4			8				
12	3		3*	0				
13	3	0						
14	1*		1					
15	1		2					
16	1							0

Asterix (*) indicates advanced polyp features such as size ≥ 1 cm or presence of villous histopathology, high grade dysplasia, or carcinoma in situ.

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